Three-dimensional, transgenic cell models to quantify space genotoxic effects

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The environment inside a spacecraft may contain radiation and chemical agents known to be mutagenic and carcinogenic in humans. Additionally, microgravity is a complicating factor that may modify or synergize induced genotoxic effects. In vitro models have advanced many aspects of our understanding of genotoxicity. The sensitivity of these models may be significantly increased in cell culture systems that provide dynamic intercellular interactions and tissue morphogenesis, thus providing a more robust platform for assessing the outcome of exposure to multiple stressors.

A state-of-the-art, three-dimensional, multicellular tissue equivalent cell culture model will be presented. It consists of mammalian cells genetically engineered to contain multiple copies of defined target genes for genotoxic assessment. NASA-designed bioreactors are used to coculture human mammary epithelial cells (H184B5) and Stratagene's (Austin, Texas) Big Blue ^{TM} Rat 2 lambda fibroblasts. The fibroblasts were genetically engineered to contain a high-density target gene for mutagenesis (60 copies of lacI/LacZ per cell). Tissue equivalent spheroids are routinely produced by inoculation of 2 to 7 X 10^5 fibroblasts with Cytodex 3 beads (150 micrometers in diameter), at a 20:1 cell:bead ratio, into 50-ml HARV bioreactors (Synthecon, Inc.). Fibroblasts are cultured for 5 days, an equivalent number of epithelial cells added, and the fibroblast/epithelial cell coculture continued for 21 days. Three-dimensional spheroids with diameters ranging from 400 to 600 micrometers are obtained. Histological and immunohistochemical characterization reveals i) both cell types present in the spheroids, with fibroblasts located primarily in the center, surrounded by epithelial cells; ii) synthesis of extracellular matrix; and iii) mitotic cells located throughout the spheroids. Spheroidal integrity and cell viability were retained for the 30-day test period after removal of spheroids from the bioreactor.

Potential utility of this three-dimensional, transgenic model for genotoxicity was initially assessed by exposure of spheroids to 0-2 Gy neon at dose rates of 0.3 to 1.5 Gy/min (National Institute of Radiological Sciences, Chiba, Japan). Quantification of mutation at the lacI gene revealed a linear dose response for mutation induction. Limited sequencing analysis of mutant clones revealed higher frequencies of deletions and multiple base sequence changes with increasing dose. These results suggest that our three-dimensional, transgenic model provides a platform for the quantification, identification, and characterization of genotoxicity incurred in space and on Earth. Furthermore the model uniquely allows investigation of cell/cell interactions, the mechanistic interaction of microgravity with radiation insults, and DNA repair. Using this three-dimensional model will allow us to obtain dual genotoxic information (i.e., mutation rate plus chromosome

aberration data) from the same system so that one endpoint can be used to reference the other, thereby increasing the fidelity of the data set. Moreover, the tissue-equivalent nature of the three-dimensional model provides high confidence for relevance of risk assessment, i.e., the establishment of quality factors directly applicable to the microgravity environment. (Supported by NASA NRA 94-OLMSA-02)